IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F1)

In re Application of: Welcher et al.	)	
	)	
Serial No.: 11/200,389	)	**Before the Examiner: J. Seharaseyon**
	)	
Filed: August 8, 2005	)	**Group Art Unit: 1647**
	)	
For: Interferon-like Molecules and Uses Thereof	)	**Confirmation No.: 3469**
	)	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE TO OFFICE ACTION MAILED NOVEMBER 23, 2007

Responsive to the Office Action mailed November 23, 2007, Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

Amendments to the Specification: Pursuant to 37 C.F.R. § 1.121, Applicants present the amendments to the specification at page 2, marked up to show changes made relative to the immediate prior version of the specification.

Amendments to the Claims: Pursuant to 37 C.F.R. § 1.121, Applicants present a complete listing of the claims, including marked up versions of all currently amended claims, at pages 3-5

Remarks: Applicants' Remarks begin on page 6 of this paper.

Amendments to the Specification under 37 C.F.R. § 1.121

Please amend the specification at page 1, line 1 as follows:

INTERFERON-LIKE ~~MOLECULES~~ PROTEINS AND USES THEREOF

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Amendments to the Claims under 37 C.F.R. § 1.121

Claim 1 (original): An isolated polypeptide comprising an amino acid sequence:

(a) as set forth in SEQ ID NO: 5; or

(b) encoded by the DNA insert in ATCC Deposit No. PTA-976.

Claim 2 (currently amended): An isolated polypeptide comprising:

~~(a) an~~ the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine~~;~~

~~(b) an amino acid sequence that is at least about 70 percent identical to the amino acid sequence set forth in SEQ ID NO: 5, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation; or~~

~~(c) a fragment of the amino acid sequence set forth in SEQ ID NO: 5 comprising at least about 25 amino acid residues, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation, or is antigenic~~.

Claim 3 (cancelled).

Claim 4 (currently amended): An isolated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence:

(a) as set forth in SEQ ID NO: 4;

(b) of the DNA insert in ATCC Deposit No. PTA-976; or

(c) encoding a polypeptide as set forth in SEQ ID NO: 5~~; or~~

~~(d) that hybridizes to the complement of the nucleotide sequence of any of (a)-(c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences;~~

~~wherein the encoded polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation~~.

Claims 5-7 (cancelled).

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Claim 8 (currently amended): A composition comprising the polypeptide of any of Claims 1, 2, or ~~3~~ 4, and a pharmaceutically acceptable formulation agent.

Claim 9 (original): The composition of Claim 8, wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-oxidant.

Claim 10 (currently amended): The composition of Claim 8, wherein the polypeptide comprises ~~an~~ the amino acid sequence as set forth in SEQ ID NO: 6.

Claim 11 (currently amended): A polypeptide comprising a derivative of the polypeptide of any of Claims 1, 2, or ~~3~~ 4.

Claim 12 (original): The polypeptide of Claim 11 that is covalently modified with a water-soluble polymer.

Claim 13 (original): The polypeptide of Claim 12, wherein the water-soluble polymer is polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxidelethylene oxide copolymers, polyoxyethylated polyols, or polyvinyl alcohol.

Claim 14 (currently amended): A fusion polypeptide comprising the polypeptide of any of Claims 1, 2, or ~~3~~ 4 fused to a heterologous amino acid sequence.

Claim 15 (original): The fusion polypeptide of Claim 14, wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

Claim 16 (currently amended): A polypeptide produced by a process comprising culturing a host cell comprising a vector comprising a nucleic acid molecule comprising a nucleotide sequence:

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(a) as set forth in SEQ ID NO: 4;

(b) of the DNA insert in ATCC Deposit No. PTA-976; or

(c) encoding a polypeptide as set forth in SEQ ID NO: 5; ~~or~~

~~(d) that hybridizes to the complement of the nucleotide sequence of any of (a)-(c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences,~~

~~wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;~~

under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

Claims 17-18 (cancelled).

Claim 19 (currently amended): The polypeptide of ~~any of~~ Claim[[s]] 16~~, 17, or 18~~, wherein the host cell is a eukaryotic cell.

Claim 20 (currently amended): The polypeptide of ~~any of~~ Claim[[s]] 16~~, 17, or 18~~, wherein the host cell is a prokaryotic cell.

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REMARKS

Claims 2, 4, 8, 10, 11, 14, 16, 19, and 20, as amended, and claims 1, 9, 12, 13, and 15 are pending in the instant application. Claims 3, 5-7, 17, and 18 have been canceled without prejudice or disclaimer. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

1. Claim of priority

The Office Action states that the instant application appears to claim subject matter disclosed in U.S. Application No. 09/927,850 (the '850 application), and that Applicants must amend the specification to insert a reference to the '850 application as the first sentence of the specification if Applicants intend to rely on the filing date of the '850 application under 35 U.S.C. §§ 119(e), 120, 121, or 365(c).

Coinciding with the submission of this Response, Applicants have submitted a Petition for an Unintentionally Delayed Domestic Priority Claim in order to amend the first sentence of the instant application to insert a reference to the '850 application.

2. Objections to the Specification

The Office Action contains an objection to the specification because the title of the invention is not descriptive. The Action states that a new title is required that is clearly indicative of the invention to which the claims are directed.

Applicants have amended the title to read: "Interferon-Like Proteins and Uses Thereof," which Applicants contend is clearly indicative of the invention to which the claims are directed. Applicants, therefore, respectfully request that this ground of objection be withdrawn.

The Office Action asserts an objection to the specification because of the improper use of the trademark "Quick Spin" and "Taq polymerase," which the Action states should be capitalized and accompanied by generic terminology.

Applicants have searched the U.S. Patent and Trademark Office Trademark Electronic Search System (TESS), but have not found a record for the term "Quick Spin" with respect to

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Qiagen's G-50 column. Applicants were also unable to find a record for the term "Taq polymerase." Applicants contend that because the terms "Quick Spin" and "Taq polymerase" do not appear to be registered trademarks, these terms do not to be capitalized or accompanied by generic terminology. Applicants respectfully request that this ground of objection be withdrawn.

3. Rejection of claims 5, 6, and 16-20 under 35 U.S.C. § 112, second paragraph

Claims 5, 6, and 16-20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicants regard as the invention.

a. Hybridization conditions

Claims 5, 6, and 16-18 are rejected for reciting the term "hybridization conditions," which the Action states is insufficiently defined in the specification.

Applicants have cancelled claims 5, 6, 17, and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites hybridization conditions, Applicants respectfully request that this ground of rejection be withdrawn.

b. Polypeptide production

Claims 16-18 are rejected for being indefinite since it is unclear how the polynucleotide complements of claims 16(d), 17(c), and 18(b) can encode a polypeptide having the activity of the polypeptides disclosed in the specification.

Applicants have cancelled claims 17 and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites subpart (d), Applicants respectfully request that this ground of rejection be withdrawn.

c. IFN-L polypeptide

Claims 2, 5, and 17 are rejected as being vague and indefinite for the reciting the term "at least about."

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Applicants have cancelled claims 5 and 17, and have amended claim 2 to delete subpart (b). As claim 2 no longer recites the term "about," Applicants respectfully request that this ground of rejection be withdrawn.

Applicants submit that the claims as amended are in condition for allowance and respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

4. Rejection of claims 1-20 under 35 U.S.C. § 112, first paragraph

a. Rejection of claims 1-20 under the enablement requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 are rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification such that one of skill in the art could make and use the invention as claimed. The Action specifically asserts that all possible variants of SEQ ID NO: 5 are not enabled, and that the amount of experimentation required to determine these variants would be undue.

Applicants respectfully disagree with the Action's assertion that the claimed variants of SEQ ID NO: 5 are not enabled. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite variants of SEQ ID NO: 5, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

The Action also asserts that phrases such as "an amino acid sequence" and "a nucleic acid molecule" in the claims read upon various variants and fragments. Applicants have cancelled claim 6 and 18, and have amended claims 2(a) and 10 to recite "the" rather than "a" or "an." Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

The Action further asserts that Applicants' referral to the deposit of PTA-976 in the specification and in claims 1, 4, 5, 16 and 17 is an insufficient assurance that all of the conditions of 37 C.F.R §§ 1.801-1.809 have been met. The Action states that Applicants must submit a statement

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300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

by an attorney of record over his or her signature, stating that a deposit has been made under the terms of the Budapest Treaty and that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent. The Action further states that the instant specification must be amended to recite the date of the deposit and the complete name and address of the depository, and that the claims must be amended to recite the accession number.

Pursuant to the Examiner's request, Applicants' representative submits the following statement: Applicants deposited cDNA encoding human IFN-L polypeptide with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. The deposit was accepted by the ATCC, an International Depository Authority, under the provisions of the Budapest Treaty, and the deposit was designated as PTA-976. A copy of the ATCC receipt for this deposit, showing the patent deposit designation (Accession No. PTA-976) and the date on which the deposit was received by the ATCC (November 23, 1999), is attached. Pursuant to 37 C.F.R. § 1.808(a)(2), the deposit was made under conditions that assure that all restrictions imposed by the depositors on the availability to the public of the deposited material would be irrevocably removed upon the granting of a patent relying on the deposited biological material. In making the deposit, Applicants acknowledged their responsibility, pursuant to 37 C.F.R. § 1.805, to provide a replacement or supplemental deposit if the depository possessing the deposit is unable to furnish samples thereof or is able to furnish samples thereof but the deposit has become contaminated or has lost its capability to function as described in the specification. With regard to the assertion that the date of the deposit and the complete name and address of the depository is not referred to in the body of the specification, Applicants respectfully direct the Examiner's attention to page 92, lines 25-28 of the specification as-filed, where Applicants disclose that a deposit of cDNA encoding human IFN-L polypeptide, subcloned into pSPORT1 (Gibco BRL) and transfected into *E. coli* strain DH10B, having Accession No. PTA-976, were made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on November 23, 1999. With regard to the assertion that the accession number of the deposit is not referred to in the claims, Applicants respectfully direct the Examiner's attention to claims 1(b), 4(b), and 16(b), as originally filed. Applicants contend that all the requirements of 37 C.F.R. §§ 1.801-1.809 have been met. *In re*

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300 South Wacker Drive
Chicago, Illinois 60606
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Lundak, 225 U.S.P.Q. 90 (Fed. Cir. 1985). Withdrawal of this rejection is therefore respectfully solicited.

Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejections under the enablement requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

b. Rejection of claims 1-20 under the written description requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 have been rejected as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Action asserts that the specification does not disclose all possible variants of nucleic acid molecules that hybridize to the complement of the claimed nucleotide sequences with more than 21% mismatch. The Action also asserts that specification has failed to disclose any other sequence contemplated in the instant claims including IFN-L fragments and other variants, and thus the skilled artisan cannot envision the detailed chemical structures of the claimed polypeptide sequences. Finally, the Action asserts that the species specifically disclosed are not representative of the genus because the genus is highly variant.

Applicants respectfully disagree with the Action's assertion that the specification does not contain an adequate written description of the claimed invention. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite the variants described above, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

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Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejection under the written description requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

5. Rejection of claims 1-20 under 35 U.S.C. § 102(e)

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,433,145 (the '145 patent).

Pursuant to 37 C.F.R. § 41.202, Applicants suggest that an interference be declared between the instant application and U.S. Patent No. 6,433,145 (the '145 patent). The '145 patent issued on August 13, 2002 from U.S. Application No. 09/487,792 (the '792 application), which was filed on January 20, 2000. The '792 application is a continuation-in-part of U.S. Application No. 09/358,587 and International Application No. PCT/US99/16424, both filed on July 21, 1999, and claims the benefit of U.S. Provisional Application No. 60/093,643, filed July 21, 1998.

Applicants believe claims 1 and 51 of the '145 patent interfere with claims 1 and 2 of the instant application. Claims 1 and 51 of the '145 patent read as follows:

> 1. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:
> (a) amino acids 1 to 207 of SEQ ID NO:2;
> (b) amino acids 7 to 207 of SEQ ID NO:2;
> (c) amino acids 2 to 207 of SEQ ID NO:2; and
> (d) amino acids 28 to 207 of SEQ ID NO:2.
>
> 51. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:
> (a) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity;
> (b) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment inhibits bone marrow proliferation;
> (c) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment activates the Jak/Stat pathway; and
> (d) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

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300 South Wacker Drive
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312-913-0001

Claims 1 and 2 of the instant application read as follows:

1. An isolated polypeptide comprising an amino acid sequence:
   (a) as set forth in SEQ ID NO: 5; or
   (b) encoded by the DNA insert in ATCC Deposit No. PTA-976.

2. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine.

Applicants propose the following counts:

1. An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2 of U.S. Patent No. 6,433,145.

2. An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

A comparison between the proposed counts, claims 1(a) and 51 of the '145 patent, and claims 1(a) and 2 of the instant application is provided in the claim chart below:

Count 1	**Claim 1(a) of '145 patent**	**Claim 1(a) of the instant application**
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	having an amino acid sequence [that is] amino acids 1 to 207 of SEQ ID NO:2[.]	comprising an amino acid sequence . . . as set forth in SEQ ID NO: 5 . . . .
Count 2	**Claim 51 of '145 patent**	**Claim 2 of the instant application**
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid	having an amino acid	comprising the amino acid

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sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence [that is] the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence as set forth in SEQ ID NO: 6 . . . .
wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	wherein the fragment has anti-viral activity; . . . wherein the fragment inhibits bone marrow proliferation; . . . wherein the fragment activates the Jak/Stat pathway; [or] wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	[While claim 2 does not expressly recite this limitation, the polypeptide recited in the claim, which is the mature form of the full-length IFN-L polypeptide, would inherently have such properties.]

The sequence alignment provided in Exhibit A indicates that the amino acid sequence of SEQ ID NO: 5 of the instant application shares 100% sequence identity with amino acids 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 5 of claim 1(a) of the instant application and amino acids 1 to 207 of SEQ ID NO: 2 of claim 1(a) of the '145 patent share 100% identity, the subject matter of either claim would, if prior art, have anticipated the subject matter of the other claim.

The sequence alignment provided in Exhibit B indicates that the amino acid sequence of SEQ ID NO: 6 of the instant application shares 100% sequence identity with amino acids 30 to 207 of SEQ ID NO: 2 of the '145 patent. The amino acid sequence of SEQ ID NO: 6 is, therefore, a fragment of amino acid residues 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 6 is the mature form of the amino acid sequence of SEQ ID NO: 2 of the '145 patent (as well as of the amino acid sequence of SEQ ID NO: 5 of the instant application), the amino acid sequence of SEQ ID NO: 6 would have anti-viral

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activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2. Applicants also contend that because the amino acid sequence of SEQ ID NO: 6 of claim 2 of the instant application shares 100% identity with a fragment of the amino sequence of SEQ ID NO: 2 of the '145 patent, and the mature form would have anti-viral activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2, the subject matter of either claim 51 of the '145 patent and claim 2 of the instant application would, if prior art, have anticipated the subject matter of the other claim.

Applicants contend that the Declaration Pursuant to 37 C.F.R. § 1.131 which is provided in Exhibit C, and which was submitted on June 24, 2004 for U.S. Application No. 09/927,850 (from which the instant application claims the benefit of priority as a continuation application), establishes that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared. In particular, the Declaration provides copies of forty-one (41) pages from the inventors' laboratory notebook showing conception of the claimed invention before July 21, 1998. The laboratory notebook pages show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook pages). Specifically, three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (clones 2, 6, and 7; *see* page 40 of laboratory notebook pages). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62 of laboratory notebook pages). The amino acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

The Declaration also provides copies of ten (10) pages from a Research Summary prepared by the inventors showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the Research Summary shows that the inventors performed experiments in order to determine the function of protein encoded by the nucleic acid sequence described above, and that once the function of the protein had been determined, the inventors prepared a Research Summary and submitted that Summary to the legal department of

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300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

Amgen Inc., the assignee of the instant application. More particularly, the Research Summary shows that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7 of Research Summary) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10 of Research Summary). Thus, the Declaration and attachments provided in Exhibit C establish that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared.

Applicants note that claims 1(a) and 2 of the instant application were not added or amended in order to provoke an interference. Applicants contend, therefore, that pursuant to 37 C.F.R. § 41.202(a)(5), a claim chart showing the written description for claims 1(a) and 2 in the specification need not be provided.

Finally, Applicants submit the following chart showing where the instant disclosure provides a constructive to reduction of practice within the scope of the interfering subject matter:

Count 1	**Reduction to Practice in Instant Disclosure**
An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	page 4, lines 9-12; page 7, lines 28-30; page 11, lines 4-5; page 95, lines 10-16; and Figures 2A-2B.
Count 2	**Reduction to Practice in Instant Disclosure**
An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity.	page 4, lines 16-19; page 12, line 28 to page 13, line 3; page 7, lines 28-30; page 95, lines 10-16; page 103, lines 20-27; and Figures 2A-2B.

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300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

In view of the above discussion, Applicants respectfully suggest that an interference be declared between the instant application and the '145 patent.

CONCLUSIONS

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

If Examiner Seharaseyon believes it to be helpful, he is invited to contact the undersigned representative by telephone at 312-913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff LLP

Dated: December 23, 2008

By: /Donald L. Zuhn, Jr./
Donald L. Zuhn, Jr., Ph.D.
Reg. No. 48,710

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

Exhibit A

```
                    10         20         30         40         50         60
                     |          |          |          |          |          |
SEQID2       MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
SEQID5       MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
             ************************************************************
Prim.cons.   MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL

                    70         80         90        100        110        120
                     |          |          |          |          |          |
SEQID2       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
SEQID5       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
             ************************************************************
Prim.cons.   RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ

                   130        140        150        160        170        180
                     |          |          |          |          |          |
SEQID2       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
SEQID5       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
             ************************************************************
Prim.cons.   QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD

                   190        200
                     |          |
SEQID2       CAWEIVRVEIRRCLYYFYKFTALFRRK
SEQID5       CAWEIVRVEIRRCLYYFYKFTALFRRK
             ***************************
Prim.cons.   CAWEIVRVEIRRCLYYFYKFTALFRRK
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Exhibit B

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                    10         20         30         40         50         60
                     |          |          |          |          |          |
SEQID2         MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
SEQID6         ------------------------------CNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
                                             ******************************
Prim.cons.     MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL

                    70         80         90        100        110        120
                     |          |          |          |          |          |
SEQID2         RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
SEQID6         RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
               ************************************************************
Prim.cons.     RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ

                   130        140        150        160        170        180
                     |          |          |          |          |          |
SEQID2         QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
SEQID6         QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
               ************************************************************
Prim.cons.     QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD

                   190        200
                     |          |
SEQID2         CAWEIVRVEIRRCLYYFYKFTALFRRK
SEQID6         CAWEIVRVEIRRCLYYFYKFTALFRRK
               ***************************
Prim.cons.     CAWEIVRVEIRRCLYYFYKFTALFRRK
```


McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

Exhibit C

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

IN THE UNITED ST TES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of: Welcher al.	)	
	)	
Serial No.: 09/927,850	)	Before the Examiner: J. Andres
	)	
Filed: August 10, 2001	)	Group Art Unit: 1646
	)	
For: Interferon-Like Molecule and Uses Thereof	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARA' ON PURSUANT TO 37 C.F.R § 1.131

We, Andrew A. Welcher, r iding at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, T ousand Oaks, California; and Michael Kelley, residing at 3866 Alta Mesa Drive, Studio City, Cal ornia; hereby declare:

1. We are named co-i ventors on United States Application No. 09/927,850, filed on August 10, 2001.

2. The invention disc sed and claimed in the instant patent application was conceived in the United States by us before ly 21, 1998 and was then diligently reduced to practice.

3. Accompanying thi Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing con ception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory not ook show that a genomic cloning approach was used to identify the nucleic acid sequence of huma interferon-like polypeptide (*see* page 34 of laboratory notebook). Three genomic clones were ider fied as containing nucleic acid sequences encoding at least a portion of human interferon-like p lypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were olated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was termined to contain a partial nucleic acid sequence for human interferon-like polypeptide and a other genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence or human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon ike polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the iboratory notebook pages have been redacted from the photocopies. However, the dates e before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was f d, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanyin this Declaration are photocopies of ten (10) pages from a Research Summary showing tha the invention disclosed and claimed in the instant patent application was diligently reduce to practice. Specifically, the photocopies of the Research Summary show that experiments ere performed in order to determine the function of protein encoded by the nucleic acid sequer e described in paragraph 3 above, and that once the function of the protein had been determined, Research Summary was prepared and submitted to the legal department of Amgen Inc., the assi nee of the instant application. More particularly, photocopies of the Research Summary show tha several versions of the human and rat IFN-L proteins were produced in a mammalian expres on system (*see* page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines wa found to cause phosphorylation of cellular proteins (*see* page 10).

6. The dates on th Research Summary pages have been redacted from the photocopies.

7. We hereby declare urther that all statements made herein by each of us to our own knowledge are true and that all st ements made on information and belief are believed to be true; and further that these statements v re made with the knowledge that willful false statements and the like so made are punishable by fi or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that su willful false statements may jeopardize the validity of the application or any patent issuing ereon.

Dated: June 10, 2004

Signed: Andrew A. Welcher
Andrew A. Welcher

Duanzhi Wen
Duanzhi Wen

Michael Kelly

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of:	**Welcher et al.**	)
Serial No.:	**09/927,850**	) **Before the Examiner:** **J. Andres**
Filed:	**August 10, 2001**	) **Group Art Unit:** **1646**
For:	**Interferon-Like Molecules and Uses Thereof**	)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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6. The dates on the Research Summary pages have been redacted from the photocopies.

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Dated: June 25, 2004

Signed: _____
Andrew A. Welcher

_____
Duanzhi Wen

Michael Kelly

ge No.

[redacted] hrpe3-00078-F6.

② related to rIFNβ (30%).

③ ⊕ in certain lot of pancreas mRNA(human) Northern (J. Cao).

③ Screening of pancreas cDNA(human) library ⊖.

? Different lot of RNA source

? Different level of expression

Decision: ① re-screening human pancreas library

② determine the genomic screening condition

③ Is genomic screening feasible?

[redacted] IFN probe for genomic blot

PAGE: 1

[redacted] 13:16

ID: CHERENCOV 0.5
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H#	: NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC#	:YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC	: NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX:	NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:				none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IG#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	655.1	427775.8	0.43	0.00	0.83

4.3×10^5 cpm/λ

To Page No.

d & Understood by

Date

Page No. [redacted]

PCR - Hot rTFN probe:

template	1λ
1795-01	20pm
1795-02	1λ
10X PCR Buf.	10λ
10mM dNTPs	10λ
32P-dCTP	5λ
25mM MgCl2	16λ
Taq	1λ
H2O to	100λ

94°C 30sec, 50°C 30sec 74°C 1min for 40 cycles.

G-50 column purified.

count: 4.3×10^5 cpm/λ

Determine the hybridization and washing condition for genomic screening.

① very likely the homology of hTFN vs. rTFN is in the neighborhood of 60%.

③ The formamide should be between 25% – 30%

Washing condition should start with gentle wash, then elevate the T wash. determine a condition in which fragment is detectable while background is minor.

To Page No.

lessed & Understood by me,	Date	Invented by	Date

From the genomic southern (D. Wen keeps all the photo) it is clear.

1) there is an 1.8kb HindIII fragment that strongly hybridize w/ Probe.

2) The formamide concentration can be adjusted to ~30% for relatively optimal signal vs. noise ratio.

3) Washing can be conducted @ ~55°C in 0.2 OR 0.3X SSC, 0.1% SDS.

Wen further found in literature:

1) IFN λ family member is single gene, i.e. no intron.

2) A lot of pseudo IFN gene as well.

But what about we identify a gene resemble pmpe3-0078-F6, also proved its expressing tissues?

[redacted] Screening h.Pancreas Library w/ Rat Probe

IFN - probe for human pancreas Library screening PAGE: 1

[redacted] 10:24

ID: CHERENCOV 0.5
USER: 2 COMMENT: '

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	606.2	827666.2	0.31	0.01	0.91

PCR as on p.35.
Library Screening
- 1X10^6 clone on 2 plates.
- 30% Formamide. 5xSSC. 42°C O/N
- Wash. 2X SSC. 0.1% SDS 30min @ 50°C
- O/N exposure

And
There is no double positive clones.

e No.

[redacted] Screening human fibroblast genomic Library.

Library: human lung fibroblast genomic library in FIX II vector (Stratagene).

1×10^6 independent clones on 10 NITRO-cellulose membrane.

PCR probe. as described on p.35

1/98
IFN probe for genomic Screening

PAGE: 1

D: CHERENCOV 0.5 [redacted] 12:47
ER: 2 COMMENT:

ESET TIME :	0.50						
TA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
UNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
O PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
INTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
W LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

OTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

M	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
O							
f	**-1	0.50	632.0	704931.5	0.34	0.01	0.89

Lift Library to Nitrocellulose membrane.

① 5 min denature, 5 min neutralization. 15 sec 2X SSC

② 80°C bake 1 hr.

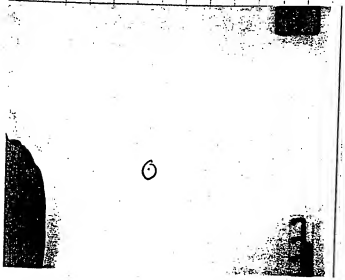
③ pre-hyb: 30% Formamamide
5X SSC
2X Denhart's
100 µg/ml ssDNA
0.1% SDS
2mM EDTA
0.1% Pyrophosphate

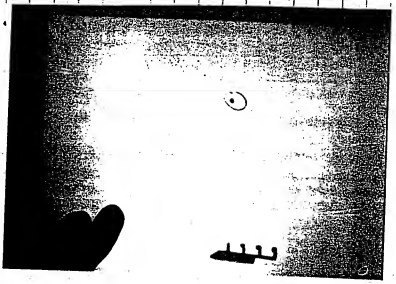
42°C O/N

④ Wash:
At R.T. 1X SSC, 0.1% SDS. 2X 30 min
At 55°C 0.2X SSC, 0.1% SDS 15 min
-80°C BioMax Kodak film O/N at -80°C

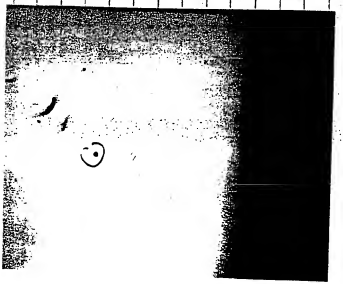
Page No.

Primary screening Result.





#2



#6

① pick up the ϕ colony with SM elution 37°C 2hrs.

② 1/500 dilution for secondary

③ double-lift secondary screening membrane.

#7. All double positive

Page No.

[redacted] 2nd screening > 6 ZFN

4/19/98
ZFN probe

PAGE: 1

ID: CHERENCOV 0.5 [redacted] 20:13
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

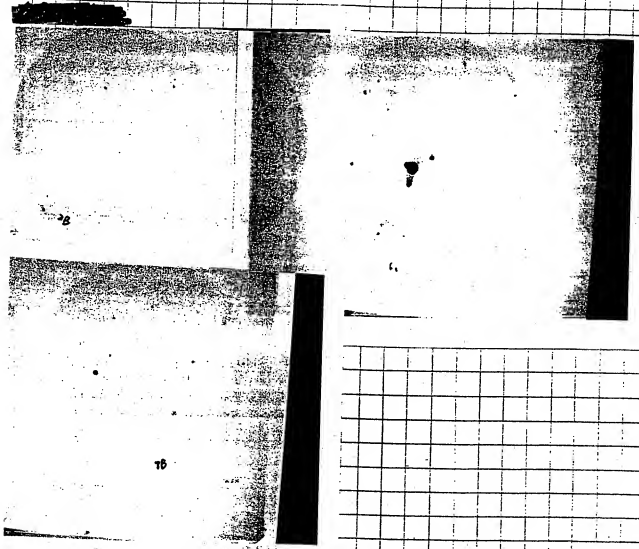
ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	615.7	458314.7	0.42	0.01	0.86

Hybridization: 20% formamide
5xSSC
42°C o/N

Wash: 1xSSC, 0.1% SDS R.T. 2min
0.2xSSC, 0.1%SDS 55°C 15min

essed & Understood by me, | Date | Invented by | Date



pick-up 2nd clone. elute with SM.
prepare for phage DNA preparation.

[redacted] Φ DNA preparation protocol,

aration of lamdan phage DNA
row up the phage (on plates or in liguid medim).
llecte the supernantat and spin down with 3ooorpn.
dd DNaseI (10u/ml) and RNaseI (20mg/ml)incubate 30 min at
c degree.
d 1/3 volume 30%PEG 6000 in3M NaCL put in ice for 1 hr or at 4
degree overnight.
10000g centrifugation 20 min
iscard supernatant and interverte the tube on papers to remove
G completely.
d 1/10-1/5 of origin volume T10E1 to suspend the phage
ticles.
d 0.5M EDTA to a final concentration of20mM and proteinaseK
mg/ml ,incubate 1hr in 55c degree waterbath.
id 5%CATBin 0.5M NaCL to a final concentration of 0.1% and put
65c degree for 5 min.
:in down and dissolve in 1.2M NaCL .
\dd 2.5vol.of enthanol and spin down.
liscard the enthanol and wash once with 70% enthanol.
.r dry the pellets and dissolve in T10E1 in ice 30 min. then at
ic degree for 10min. don,t put on RT. stock in 4c degree.

- phage has to be amplified once before proceeding to Φ DNA prep. on a 100mm plate. 10µl Φ + top agar → o/N 37°c. SM elute
- on LB / agarose plate, grow Φ o/n @ 37°C
- elute w/ SM 2 1/2 hrs @ 37.

nessed & Understood by me, | Date | Invented by | Date

e No. [redacted]

BECKMAN DU-600

Human Genomic IFN like clone

Φ prep:

Date: [redacted]
Time: 23:19

Leic Acid
adSamples

Method SaveClear Print Quit

esults file: A:\WORK_RES

Method name: A:\DEFAULT

ssay type: General Ratio and Concentration
ormula setup: VIEW
ampling device: None
ead average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

mple	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
#2	0.1428	0.0774	1.8453	0.5419	0.5711	0.7139
#6	0.3206	0.1775	1.8066	0.5535	1.2823	1.6029
#7	0.1731	0.0913	1.8962	0.5274	0.6922	0.8653

#2: 14.3 ng/λ
#6: 32 ng/λ
#7: 17.3 ng/λ

Set-up NotI digestion to release insert in FIX II.

20 mg Φ DNA
10X H
Not I (HC) 40u x 2.5 = 100
H2O

O/N @ 37°C incubator.

[redacted] where is the IFN+ fragment?
The two ← indicated on p.44 (insert of interest)
Repeat Not I digestion; run a small agarose 0.5% gel.
Transfer and Southern to determine the band.
Hyb: 30% formamide, wash: 0.2xSSC, 0.1%SDS.
55°C 15min.

Re-calc on p.46

BECKMAN DU-600

Date: [redacted]
Time: 16:18

ucleic Acid
ReadSamples Method SaveClear Print Quit

Results file: A:\WORK_RES
Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

1/20

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
1 #2	0.0059	0.0027	2.1876	0.4571	0.0235	0.0293
2 #6	0.0082	0.0043	1.8895	0.5292	0.0326	0.0408
3 #7	0.0085	0.0046	1.8522	0.5399	0.0338	0.0423

4

#2 5.9 x 50 x 20 ≈ 6ng/λ
#6 8.2 x 1000 = 8ng/λ
#7 8.5 x 1000 = 8.5ng/λ

Ligation: 10ng cut vect.
+ 6λ Insert
+ 2λ 10x Buff
5U T4 ligase

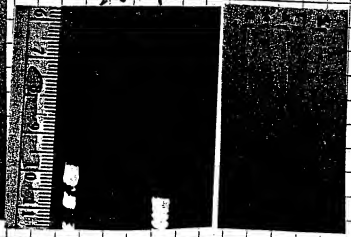
essed & Understood by me, | Date | Invented by | Date

Page No.

[redacted]

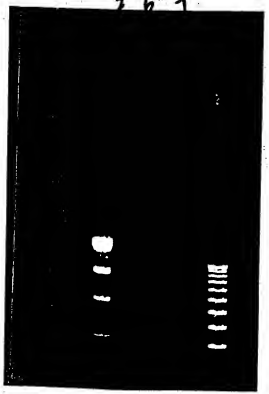
267

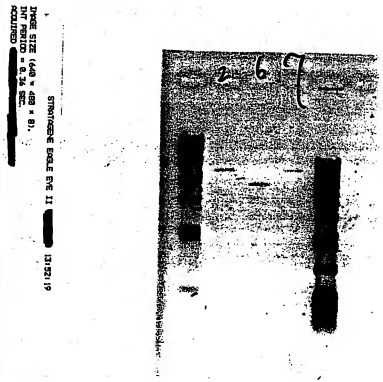
267



[redacted] Gel purify ~~Eluts~~ ITN genomic fragment

267





d & Understood by me. | Date | Invented by | Date

Page No.__

pSV. Sport/IFN genomic fragment

BECKMAN DU-600

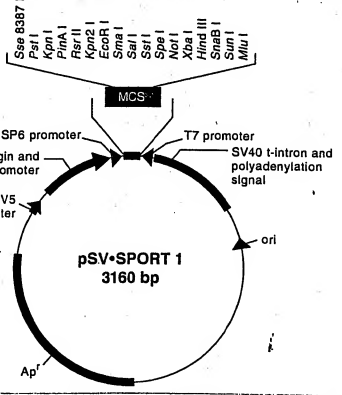
cleic Acid
eadSamples Method

Results file: A:\WORK_RES

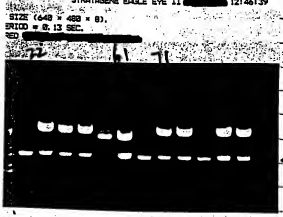
Assay type: General Ratio and Concen
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Sample ID	abs 260.0 nm	abs 280.0 nm
1 psv·psport Hind III	0.0107	0.0039
2	0.0204	0.0093
3 psv·psport Not I		

psv. psport/Not I 20 ng/λ



0.8% TAE - agarose gel / Not I digestion



Miniprep DNA 5µl
10X H 1µl
Not I 0.5µl (10u)
H2O 3.5µl
Total 10µl @ 37°C 1½hrs

To Page No.____

ssed & Understood by me,	Date	Invented by	Date

Page No.

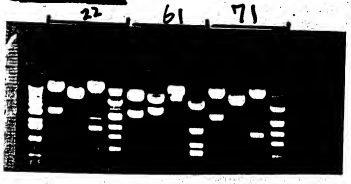
[redacted] Set-up genomic clone Analysis.

5λ mini-prep DNA
1λ 10x Buffer (BMB)
0.5λ E2
3.5λ H2O
10λ @ 37°C 1½ hrs.

Restriction E2's:
present on MCS of psv.pspart
NotI, EcoRI, XbaI & HindIII

8% TAE gel.

STRATAGENE EAGLE EYE II [redacted] 15:18:42
E (640 × 480 × 8).
D = 0.46 SEC.

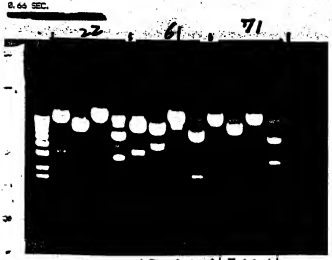


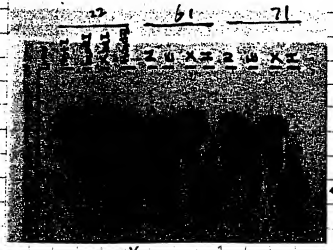
22 and 71 are identical independent clones, but not 61.

	22	61	71
EcoRI	0	2	0
XbaI	3	2	3
HindIII	≥4	≥4	≥4

Is that why initially EcoRI digestion on genomic DNA didn't light up by pmrnrep3 probe?

STRATAGENE EAGLE EYE II [redacted] 15:13:05
640 × 480 × 8).
0.46 SEC.





Transfer this gel w/ alkaline transfer.

* HindIII incomplete digestion

To Page No.

Page No.

[redacted] Washing Southern Blot.

Hybridization: 30% Formamide, 5x SSC, 2x Denhart's
100μg SSDNA, 0.2% SDS, 2mM EDTA, 0.1% pyrophos
(No NaH_2PO_4)
42°C, 3 hrs. + 5% Dextran Sulfate for Hyb.

PCR – Hot probe:

Template	1λ (20ng)
1795-01	1λ (20pm)
1795-02	1λ (20pm)
10mM dNTP	10λ (dCTP @ 0.1mM)
^{32}P-dCTP	5λ
10x PCR Buf.	10λ
25mM $MgCl_2$	16λ (4mM final)
Taq	1λ
H_2O	to 100 ul

Control: +60ul Hyb with cold dNTPs

5/7/98 RIN probe

PAGE: 1

ID: CHERENCOV 0.5
USER: 2 COMMENT: [redacted] 22:04

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	592.9	528289.8	0.39	0.01	0.86

1ul count.

Washing: • 1 X SSC, 0.1% SDS @ RT 1hr
0.2 X SSC, 0.1% SDS @ 55°C 15min
exp. @ -80°C O/N.

To Page No.

Read & Understood by me, | Date | Invented by | Date

e No.

[redacted] h mrep-3 cloning: Subclone genomic fragment.

Set-up digestion:

70λ	mini-prep DNA
8λ	10x B Buffer (BMB)
2λ	BamHI (20u)
80λ	total @ 37°C 5hrs

8λ run gel (with dye) ◀ indicates positive band in Southern Blot with mmrep-3 probe (p. 49).

92λ (with dye) run a 0.8% TAE-gel.

Qiagen Gel Purification Kit

No. 61 No. 71

0.8% TAE-agarose gel (Hind III fragments)

No. 61, 71

61, 71 insert is probably same (identical size, Hind III fragment size)

BECKMAN DU-600

Date: [redacted]
Time: 21:25

leic Acid
adSamples Method SaveClear Print Quit

esults file: A:\WORK_RSS Method name: A:\DEFAULT

ssay type: General Ratio and Concentration Units: UG/UL
ormula setup: VIEW Background Correction: [No]
ampling device: None Concentration: [Yes]
ead average time: 0.50 sec Peak Pick: [No]

1/10 dilu

mple	abs 260.0 nm	abs 280.0 nm	x100 280.0 nm	X100 260.0 nm	ssRNA UG/UL	dsDNA UG/UL
#61	0.0168	0.0093	1.7933	0.5576	0.0670	0.0838
#71	0.0201	0.0112	1.7963	0.5567	0.0803	0.1003

#61 0.0168 × 50 × 20 ≈ 17ng/λ

#71 0.0206 × 50 × 20 ≈ 20ng/λ

& Understood by me. Date Invented by

[redacted] Ligation:

3λ dephosphorylated psv-psport (17ng)
1λ 10x Lig. Buffer (BMB)
1λ T4 DNA Ligase (BMB)
5λ Insert (85 ~ 10ng)
10λ

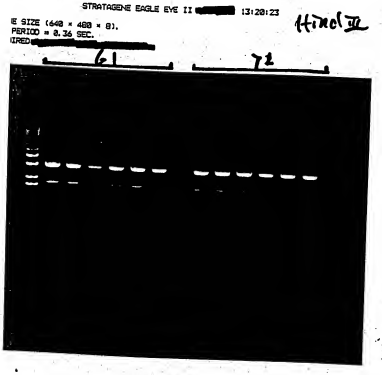
Control: +5Nl H2O in vector control.

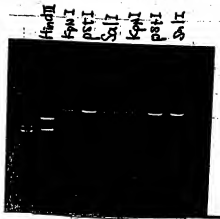
14°C 1hr

Transformation: 190λ DH10α ElectroMax cell
+ 1.5λ Ligation Mix

6 transformation clones/each for mini-prep.

Digest w (Hind III
5λ DNA
1λ 10XB (BMB)
0.5λ Hind III (5u)
3.5λ H2O
10λ total 37°C 1½hrs





a 8% TAE gel

All contain insert. (1.8Kb)

Further Analysis. w
KpNI. pstI. SalI (BMB)
5λ DNA
1λ 10x Buffer
0.5λ E2 (5u)
3.5λ H2O
10λ 37°C 1½hr

So no additional E2 cut in the insert.

Transfer to N.C. for future reference

61 71

Sequencing Request

ID	Clone	Requestor		Status	Submit	Rcvd
9808902	hgmrep3-6.1	Wen	Chen	Pending	[redacted]	00/00/00
9808903	hgmrep3-6.2	Wen	Chen	Pending	[redacted]	00/00/00
9808904	hgmrep3-6.5	Wen	Chen	Pending	[redacted]	00/00/00
9808905	hgmrep3-7.1	Wen	Chen	Pending	[redacted]	00/00/00
9808906	hgmrep3-7.2	Wen	Chen	Pending	[redacted]	00/00/00
9808907	hgmrep3-7.2 X deleted!	Wen	Chen	Pending	[redacted]	00/00/00
9808908	hgmrep3-7.3	Wen	Chen	Pending	[redacted]	00/00/00

3-6.1, 3-6.5, 3-7.1 } identical insert in same orientation

3-6.2, 3-7.2 } identical insert in reverse orientation.

[redacted] mCmp2 Section: Human Genomic DNA

probe: mCmp2 template 2λ (1ong)
(582-15' primer) 2λ (20pm)
(536-79C 3' primer) 2λ (20pm)
10 x Buffer 10λ
(1mM dATP, dCTP @ 0.1mM) 10λ
α-^{32}PdCTP 5λ
25mM $MgCl_2$ 16λ
Taq (BMB) 1λ
H_2O 52λ
100λ

Cold control + 16λ 10mM dATP + 52λ extra H_2O
94°C 30sec → 60°C 30sec → 72°C 45sec 5 cycles

PAGE: 1

ID: CHERENCOV 0.5
USER: 2 COMMENT:
[redacted] 16:06

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	602.8	1115564	0.27	0.02	0.95

1λ 1.1 x 10^6 cpm/λ

Cold control.

6/ primary Screening:

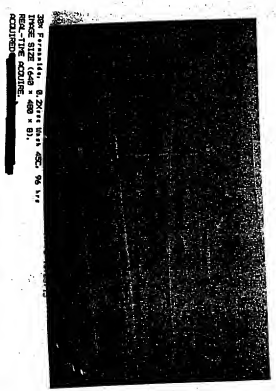
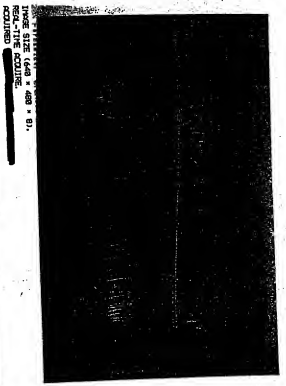
To Page No. 60

e No.

Hybridization in 30% Formamide

	Stock	Final
30% Formamide		
5×SSC		2.5ml
2× Denhart's		4ml
10μg/ml ssDNA		1ml
0.2% SDS		1ml
2mM EDTA		0.4ml
0.1% Pyrophosphate		1ml
H2O to 100 ml		37.6ml

42°C O/N . stripe rehyb. ✓

ation	30%	20%	20%
°C (Formamide)			
: SSC	0.2X	0.5X	0.5X
SDS	0.1%	0.1%	0.1%
°C exp	24 hrs	24 hrs	24 hrs

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REVERSE-COMPLEMENT of: 9808902.Con check: 3852 from: 1 to: 1163
WChen
DNA-hgmrep3-6.1 p=890-24 end
assembled by JK: [redacted]
9808902.con

With 1 enzymes: HINDIII

[redacted] 17:01 ..

```
          CTGAGAAGAGTCACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCT
        1 ---------+---------+---------+---------+---------+---------+ 60
          GACTCTTCTCAGTGGACCGTTTTAGACTCTGTAGACTCATCATACTCGTTAAGTAAAGGA

a         L  R  R  V  T  W  Q  N  L  R  H  L  S  S  M  S  N  S  F  P  -

          GTAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTGCAATACACC
       61 ---------+---------+---------+---------+---------+---------+ 120
          CATCTTACAGATGCTCTTTTGTATCGAAAACTCAACGGGGTTCTCAAAGACGTTATGTGG

a         V  E  C  L  R  E  N  I  A  F  E  L  P  Q  E  F  L  Q  Y  T  -

          CAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAATGTCCCTACAGGCCTTCAAC
      121 ---------+---------+---------+---------+---------+---------+ 180
          GTTGGATACTTCTCCCTGTAGTTCTTCCGGAAGATACTTTACAGGGATGTCCGGAAGTTG

a         Q  P  M  K  R  D  I  K  K  A  F  Y  E  M  S  L  Q  A  F  N  -

          ATCTTCAGCCAACACACCTTCAAATATTGGAAAGAGAGACACCTCAAACAAATCCAAATA
      181 ---------+---------+---------+---------+---------+---------+ 240
          TAGAAGTCGGTTGTGTGGAAGTTTATAACCTTTCTCTCTGTGGAGTTTGTTTAGGTTTAT

a         I  F  S  Q  H  T  F  K  Y  W  K  E  R  H  L  K  Q  I  Q  I  -

          GGACTTGATCAGCAAGCAGAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAAT
      241 ---------+---------+---------+---------+---------+---------+ 300
          CCTGAACTAGTCGTTCGTCTCATGGACTTGGTTACGAACCTCCTTCTGCTCTTACTTTTA

a         G  L  D  Q  Q  A  E  Y  L  N  Q  C  L  E  E  D  E  N  E  N  -

          GAAGACATGAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAGGGTCCCCCAG
      301 ---------+---------+---------+---------+---------+---------+ 360
          CTTCTGTACTTTCTTTACTTTCTCTTACTCTACTTTGGGAGTCTTCGGTCCCAGGGGTC

a         E  D  M  K  E  M  K  E  N  E  M  K  P  S  E  A  R  V  P  Q  -

          CTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTTCCTGAAAGAAAAG
      361 ---------+---------+---------+---------+---------+---------+ 420
          GACTCGTCGGACCTTGACTCCTCTATAAAGGTGTCCTATCTGTTAAAGGACTTTCTTTTC

a         L  S  S  L  E  L  R  R  Y  F  H  R  I  D  N  F  L  K  E  K  -

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      421 ---------+---------+---------+---------+---------+---------+ 480
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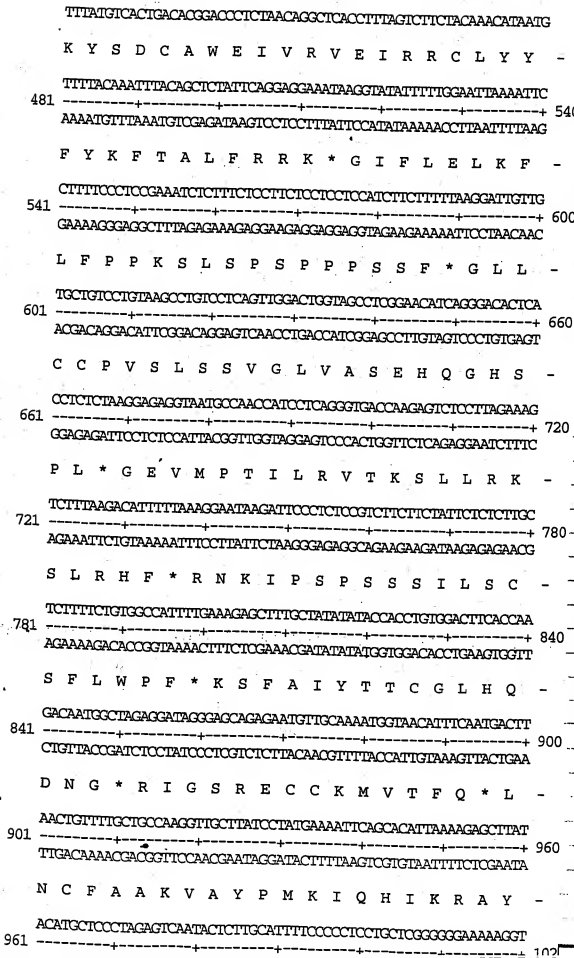
To Page No.

Date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K Y S D C A W E I V R V E I R R C L Y Y -
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481
AAAATGTTTAAATGTCGAGATAAGTCCTCCTTTATTCCATATAAAAACCTTAATTTTAAG
540
F Y K F T A L F R R K * G I F L E L K F -
CTTTTCCCTCCGAAATCTCTTTCTCCTTCTCCTCCTCCATCTTCTTTTAAGGATTGTTG
541
GAAAAGGGAGGCTTTAGAGAAAGAGGAAGAGGAGGAGGTAGAAGAAAAATTCCTAACAAC
600
L F P P K S L S P S P P P S S F * G L L -
TGCTGTCCTGTAAGCCTGTCCTCAGTTGGACTGGTAGCCTCGGAACATCAGGGACACTCA
601
ACGACAGGACATTCGGACAGGAGTCAACCTGACCATCGGAGCCTTGTAGTCCCTGTGAGT
660
C C P V S L S S V G L V A S E H Q G H S -
CCTCTCTAAGGAGAGGTAATGCCAACCATCCTCAGGGTGACCAAGAGTCTCCTTAGAAAG
661
GGAGAGATTCCTCTCCATTACGGTTGGTAGGAGTCCCACTGGTTCTCAGAGGAATCTTTC
720
P L * G E V M P T I L R V T K S L L R K -
TCTTTAAGACATTTTTAAAGGAATAAGATTCCCTCTCCGTCTTCTTCTATTCTCTCTTGC
721
AGAAATTCTGTAAAAATTTCCTTATTCTAAGGGAGAGGCAGAAGAAGATAAGAGAACG
780
S L R H F * R N K I P S P S S S I L S C -
TCTTTTCTGTGGCCATTTTGAAAGAGCTTTGCTATATATACCACCTGTGGACTTCACCAA
781
AGAAAAGACACCGGTAAAACTTTCTCTGAAACGATATATATGGTGGACACCTGAAGTGGTT
840
S F L W P F * K S F A I Y T T C G L H Q -
GACAATGGCTAGAGGATAGGGAGCAGAGAATGTTGCAAAATGGTAACATTTCAATGACTT
841
CTGTTACCGATCTCCTATCCCTCGTCTCTTACAACGTTTTACCATTGTAAAGTTACTGAA
900
D N G * R I G S R E C C K M V T F Q * L -
AACTGTTTTGCTGCCAAGGTTGCTTATCCTATGAAAATTCAGCACATTAAAAGAGCTTAT
901
TTGACAAAACGACGGTTCCAACGAATAGGATACTTTTAAGTCGTGTAATTTTCTCGAATA
960
N C F A A K V A Y P M K I Q H I K R A Y -
ACATGCTCCCTAGAGTCAATACTCTTGCATTTTCCCCCTCCTGCTCGGGGGGAAAAAGGT
961
1021

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```
a       T C S L E S I L L H F P P P A R G E K G  -

        TGACATTTCTGGCCCATTTCCTTCTCAGCTTGGTTTGTTTGAATTGATGCTTGTGTGGAATG
  1021  ---------+---------+---------+---------+---------+---------+ 1080
        ACTGTAAAGACCGGGTAAAGGAAGAGTCGAACCAAACAAACTTAACTACGAACACCTTAC

INTRON>
INTRON>
a       * H F W P I S F S A W F V * I D A C G M  -

        GTATTTCATTACTTTAAGAGTGAAGATCCATAGTGAAATTGGATGGATGGTTGAATTAGA
  1081  ---------+---------+---------+---------+---------+---------+ 1140
        CATAAAGTAATGAAATTCTCACTTCTAGGTATCACTTTAACCTACCTACCAACTTAATCT

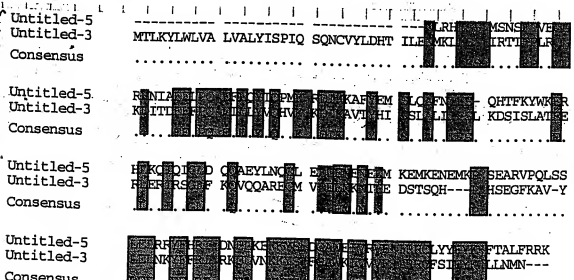
a       V F H Y F K S E D P * * N W M D G * I R  -

          HindIII
            |
        CGACCATTAAGCTTACGTACGCG
  1141  ---------+---------+--- 1163
        GCTGGTAATTCGAATGCATGCGC

a       R P L S L R T   -

Enzymes that do cut:

 HindIII
```



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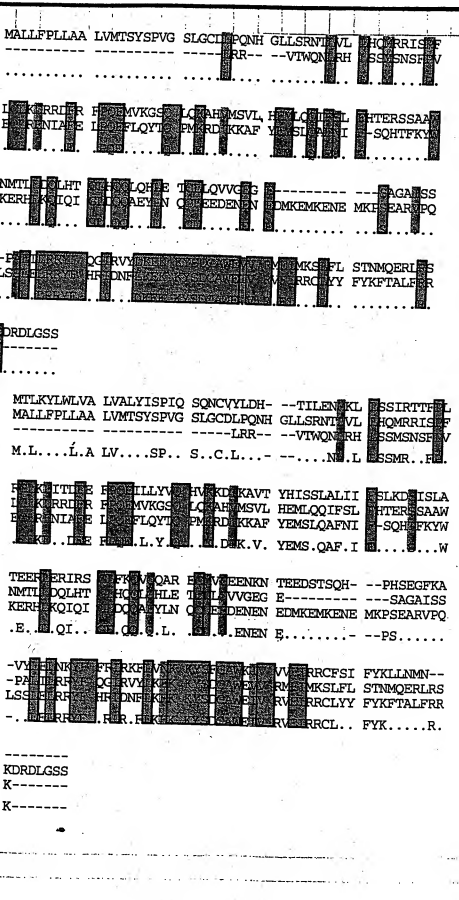
Project No._____
Book No._____ TITLE IFN

Page No.___



To Page No.____

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Match display thresholds for the alignment(s):
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9808902.Con x Mrpe3-00078-F6-Wz.Ctg [redacted] 13:52 ..

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 159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

1102 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 1053
     ||  |||||||  ||| | ||  | |||||||||  || |||||| |||||
 209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

1052 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 1003
     |  |||  ||| | | |||| | ||||||  |||   | |  |||| | ||
 259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

1002 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 956
      ||    | |||  | |  || ||||| |   || || || ||     .
 309 ATCTTCTCTGGGGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

 955 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 906
      || ||||  || | |  |||  |||  |  ||||||    | ||||
 359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

 905 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 856
      || |          | ||| |||  ||  || |||||  | |       |||
 409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

 855 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 807
      | | |       ||   |  | || | | | ||   | ||| || |  |
 453 GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

 806 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 757
      ||     | |||||||| |||  |  |||||  ||| ||    || ||
 496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

 756 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 707
      ||||  | | ||||||||||||  ||||||||||  ||||||||   ||||
 546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

 706 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 673
      ||||  ||||||||||    |  |||||||| |
 596 AAATAAGAAGATGTTTCAGTATATTTTTACAAACT 629
```


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lo.___

Gaps: 4
Quality: 1170
lity Ratio: 2.484
Similarity: 63.383
Length: 484

N> type 9808902.pair
FITT of reverse of: 9808902.Con check: 3852 from: 1 to: 1163

gmrep3-6.1 p=890-24 end
bled by JK: [redacted]
02.con

Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

INTRON::DONGYINY [redacted] 16:07:43.44
WCHEN

INTRON::JCAO [redacted] 12:24:38.19
DONGYINY . . .

ol comparison table: Gencoredisk:[Gogcore.Data.Rundata]Swgapdna.Cmp
heck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1170 Length: 484

E [redacted] rIFN Project No._____

Book No._____

6

Page No.___ / Rabbit α r IFN

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
ANIMAL#	PI [redacted]	50% Titer	[redacted]	50% Titer
#3429	5mls 30mls		30mls	
3432	5mls 30mls		30mls	
#3433	5mls 30mls		30mls	

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
ANIMAL#	PI [redacted]	50% Titer	[redacted]	50% Titer
3429	5mls 30mls	1 1500	30mls	1 2000
432	5mls 30mls	1 2500	30mls	1 3000
3433	5mls 30mls	1 5000	30mls	1 10,000

[redacted]	50% Titer			
25mls	> 1 10,000			
25mls	> 1 10,000			
25mls	> 1 10,000			

To Page No.____

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62

1.8 kb Human IFN-like clone seq.

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted]
9808903.con

With 1 enzymes: HINDIII

[redacted] 15:37 ..

HindIII

```
                                                                    CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
      1 ---------+---------+---------+---------+---------+---------+ 60
                                                                    GCGCATGCATTCGAATTAAATTGTTTTAACCTTTTTGGATTTGATATGACACGAGACCAC

                                                                    ACCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
     61 ---------+---------+---------+---------+---------+---------+ 120
                                                                    TGGATCGTTAGTTTATTAGTGTCAGTAAACCAGTTACAGATACTAATTGAGTTACTCTGT

                                                                    GGATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
    121 ---------+---------+---------+---------+---------+---------+ 180
                                                                    CCTACAAACCGATATCGTGGTCCATGTTTTTTATATAAAAGTACTTCCTAGTGAGGGAGA

                                                                    TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGACATGGACTCACAGCTTTTGGC
    181 ---------+---------+---------+---------+---------+---------+ 240
                                                                    ATACATTATCTAAACCCACTCACTCACTCACTCACTCACGTACCTGAGTGTCGAAAACCG

                                                                    TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
    241 ---------+---------+---------+---------+---------+---------+ 300
                                                                    AAAGACTTTATGGGACGTAGTCAGAACAATACTACTAAGGAATCACGACCCTACCTAGTA

                                                                    CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAG
    301 ---------+---------+---------+---------+---------+---------+ 360
                                                                    GGTCCGTAAATTCCATTGTGCTACCATTAAGAAACGAGTAAAAAGTCCCTTTTTTTTTTC

                                                                    TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAAGC
    361 ---------+---------+---------+---------+---------+---------+ 420
                                                                    AATAGTGAAGGTTTCAGCCGTATCAGTGGGCTTCATTTTTTTTTTTTTTTTTTTTTCG

                                                                    CTCAGAGGCAAAGGAAAGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
    421 ---------+---------+---------+---------+---------+---------+ 480
                                                                    GAGTCTCCGTTTCCTTTCCCCGGCGTTGGAACCAATTGACACTTTACTGCTTACTCTTTT

                                                                    CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
    481 ---------+---------+---------+---------+---------+---------+ 540
                                                                    GAGGAGGACGACTTCTATAAGTCCATATATTTCCGTGTACTTCCTTTTGAGTTTTGTAGT

                                                                    TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
    541 ---------+---------+---------+---------+---------+---------+ 600
                                                                    AACAGTATATGTGTAGAAGACCTAAAAAATCGAACGTTTTTTTTACTCGTGGTTTGGACT
                                                                    ↳ Start
                                                                    TATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
    601 ---------+---------+---------+---------+---------+---------+ 660
```

To Page

```
ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
661 ---------+---------+---------+---------+---------+---------+ 720
TAGGGACCTGACATTGAATGACTTGCAAGTGGACTCTTCTCAGTGGACCGTTTTAGACTC

ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
721 ---------+---------+---------+---------+---------+---------+ 780
TGTAGACTCATCATACTCGTTAAGTAAAGGACATCTTACAGATGCTCTTTTGTATCGAAA

TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
781 ---------+---------+---------+---------+---------+---------+ 840
ACTCAACGGGGTTCTCAAAGACGTTATGTGGGTTGGATACTTCTCCCTGTAGTTCTTCCG

CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
841 ---------+---------+---------+---------+---------+---------+ 900
GAAGATACTTTACAGGGATGTCCGGAAGTTGTAGAAGTCGGTTGTGTGGAAGTTTATAAC

GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
901 ---------+---------+---------+---------+---------+---------+ 960
CTTTCTCTCTGTGGAGTTTGTTTAGGTTTATCCTGAACTAGTCGTTCGTCTCATGGACTT

CCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
961 ---------+---------+---------+---------+---------+---------+ 1020
GGTTACGAACCTCCTTCTGCTCTTACTTTTACTTCTGTACTTTCTTTACTTTCTCTTACT

GATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
1021 ---------+---------+---------+---------+---------+---------+ 1080
CTACTTTGGGAGTCTTCGGTCCCAGGGGGTCGACTCGTCGGACCTTGACTCCTCTATAAA

CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
1081 ---------+---------+---------+---------+---------+---------+ 1140
GGTGTCCTATCTGTTAAAGGACTTTCTTTTCTTTATGTCACTGACACGGACCCTCTAACA

CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
1141 ---------+---------+---------+---------+---------+---------+ 1200
GGCTCACCTTTAGTCTTCTACAAACATAATGAAAATGTTTAAATGTCGAGATAAGTCCTC
          end
GAAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
1201 ---------+---------+---------+---------+---------+---------+ 1260
CTTTATTCCATATAAAAACCTTAATTTTAAGGAAAAGGGAGGCTTTAGAGAAAGAGGAAG

TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
1261 ---------+---------+---------+---------+---------+---------+ 1320
AGGAGGAGGTAGAAGAAAAATTCCTAACAACACGACAGGACATTCGGACAGGAGTCAACC

ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
1321 ---------+---------+---------+---------+---------+---------+ 1380
TGACCATCGGAGCCTTGTAGTCCCTGTGAGTGGAGAGATTCCTCTCCATTACGGTTGGTA

CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
1381 ---------+---------+---------+---------+---------+---------+ 1440
GGAGTCCCACTGGTTCTCAGAGGAATCTTTCAGAAATTCTGTAAAAATTTCCTTATTCTA

TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
1441 ---------+---------+---------+---------+---------+---------+ 1500
```

To Page No

Date

```
      AGGGAGAGGCAGAAGAAGATAAGAGAGAACGAGAAAAGACACCGGTAAAACTTTCTCGAA

      TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
1501  ---------+---------+---------+---------+---------+---------+ 1560
      ACGATATATATGGTGGACACCTGAAGTGGTTCTGTTACCGATCTCCTATCCCTCGTCTCT

      ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
1561  ---------+---------+---------+---------+---------+---------+ 1620
      TACAACGTTTTACCATTGTAAAGTTACTGAATTGACAAAACGACGGTTCCAACGAATAGG

      TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
1621  ---------+---------+---------+---------+---------+---------+ 1680
      ATACTTTTAAGTCGTGTAATTTTCTCGAATATGTACGAGGGATCTCAGTTATGAGAACGT

      TTTTCCCCCTCCTGCTCGGGGGGAAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
1681  ---------+---------+---------+---------+---------+---------+ 1740
      AAAAGGGGGAGGACGAGCCCCCCTTTTTCCAACTGTAAAGACCGGGTAAAGGAAGAGTCG

      TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
1741  ---------+---------+---------+---------+---------+---------+ 1800
      AACCAAACAAACTTAACTACGAACACCTTACCATAAAGTAATGAAATTCTCACTTCTAGG

                                        HindIII
                                           |
      ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
1801  ---------+---------+---------+---------+---------+---------+ 1860
      TATCACTTTAACCTACCTACCAACTTAATCTGCTGGTAATTCGAACCTAGGAGATCTCGC

      GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT
1861  ---------+---------+---------+---- 1894
      CGGCGGCTGATCACTCGAGCAGCTGGGCCCTTAA
```

Human Clone

ct No. IFN

65

10 30 50
CGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG

70 90 110
CTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA

130 150 170
ATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT

190 210 230
ATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC

250 270 290
TTCTGAAATACCCTGCATCAGTCTTGTATGATGATTCCTTAGTGCTGGGATGGATCAT

310 330 350
AGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAG

370 390 410
ATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAGC

430 450 470
CAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA

490 510 530
CCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA

550 570 590
GTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA

610 630 650
ATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTTCATTGCTGGCACCCT
MetIleGlnLysCysLeuTrpLeuGluLeuMetGlyIlePheIleAlaGlyThrLe

670 690 710
CCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
erLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr

730 750 770
ATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
HisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh

790 810 830
AGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
luLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl

850 870 890
TTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
PheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr

910 930 950
AAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGAGAGTACCTGAA
LysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGlufyrLeuAs

970 990 1010
AATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
GlnCysLeuGluGluAspGluAsnGluAsnGluAspMetLysGluMetLysGluAsnGl

1030 1050 1070
TGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
MetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh

1090 1110 1130
ACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
HisArgIleAspAsnPheLeuLysGluLysLysTyrSerAspCysAlaTrpGluIleVa

1150 1170 1190
GAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
rgValGluIleArgArgCysLeuTyrTyrPheTyrLysPheThrAlaLeuPheArgAr

1210 1230 1250
AATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
ys

1270 1290 1310
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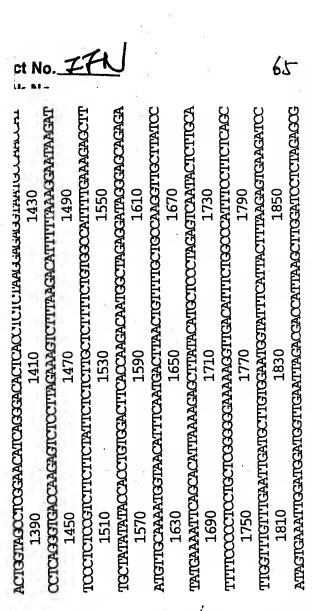

To Page No.

ZFN

rat clone

```
          10                  30                  50
GTCGACCCACGCGTCCGGGGTGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACC
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AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA
            MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer
         130                 150                 170
CCCATCCAGTCTCAGAACTGTGTGTATCTGGATCATACCATCTTGGAAAACATGAAACTT
ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu
         190                 210                 230
CTGAGCAGCATCAGGACCACCTTTCCCTTAAGATGTCTAAAAGATATCACGGATTTTGAG
LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu
         250                 270                 290
TTTCCTCAAGAGATTCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACC
PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr
         310                 330                 350
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TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla
         370                 390                 410
ACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTGCAGCAAGCTCGA
ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg
         430                 450                 470
GAGTGCATGGTAGACGAGGAGAACAAGAACACGGAGGAGGACAGTACATCACAACATCCT
GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro
         490                 510                 530
CACTCAGAGGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGA
HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg
         550                 570                 590
AAGTTCCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGGAAATA
LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle
         610                 630                 650
AGAAGATGTTTCAGTATATTTTACAAACTACTCAACATGAATTGAGAATCATCCAGCTTC
ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnEnd
         670                 690                 710
AAGCAAGAACTTAGATAGAAGTTGTGACTGCTCAAATGTCCCCAAGAACGCTTGATTCTA
         730                 750                 770
AGGCTATTGCGAGTCTGCTGCTACACACTTCGGACGCAAGACTTTTCAAGGTCAGGGTTC
         790                 810                 830
AAGGCAGTACAGTCAAAGGAAGTCTTATGTTAAGCAAAAGAAAAATTTCAGTGGAAAAGC
         850                 870                 890
TAGCAGAAATGTCAACTTGTCAAAAAAACAACTTATGGATTATGGCATTGACGTTACTAG
         910                 930                 950
CAAAAAAAATAAAACAAAAAAAAACAACAGTCACTAAAAAAAAAAAAAAAAAAGGCGGC

CGC
```

To Page

Nucleotide comparison

JPN

6

WChen
DNA-hgmrep3-6.2
assembled by JK[redacted]
9808903.con

to: Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

From: INIRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj:
From: INIRON::JCAO [redacted] 12:24:38.19
To: DONGYINY . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
CompCheck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1457 Length: 634
Ratio: 2.392 Gaps: 6
Percent Similarity: 63.516 Percent Identity: 63.516

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

9808903.Rev x Mrpe3-00078-F6-Wz.Ctg [redacted] 15:04 ..

```
555 TCTTCTGGATTTTTTAGCTT..GCAAAAAAAATGAGCACCAAACCTGATA 602
    | || | ||| ||||  |||  | | ||| | ||||||||||  |||| |
 21 TGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACCAAGGCTGAGA 70

603 TGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCT 652
    |||  | |||| ||| |||| | |  ||| ||| | ||| |||| |
 71 TGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA 120

653 GGCACCCTATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGT 702
      || ||  || | | |||||             ||  | |||
121 CCCATCCAGTCTCAGAACTGT.............GTGTATCTGGATCATAC 158

703 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 752
    || || | ||||  ||| || |||||| || || || |   | |||||
159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

753 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 802
    ||  |||||||  ||| | ||  | |||||||||| || |||||| |||||
209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

803 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 852
    |  |||  ||| | | |||| | |||||| |||   | |  |||| | ||
259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308
```

To Page N

```
853 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 899
    ||     | |||  | |  || |||||  |   || || || ||
309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

900 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 949
    || |||| || | |  |||  |||  |   ||||||    | ||||
359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

950 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 999
    || |       | ||| |||  || || |||||  | |      |||
409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

1000 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 1048
     | | |      ||   |  || | | | ||   |  ||| || |  |
453  GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

1049 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 1098
     ||     | ||||||| |||  | ||||||  ||| ||    || ||
496  AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

1099 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 1148
     ||||  | | ||||||||||||  |||||||||  ||||||||   ||||
546  CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

1149 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 1182
     |||| ||||||||||   |  |||||||| |
596  AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629
```

IFN

Project No.
Book No.

protein sequence comp

BESTFIT of: 9808903.Pep check: 4904 from: 1 to: 201

TRANSLATE of: 9808903.rev check: 8672 from: 602 to: 1205
generated symbols 1 to: 201.
REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted] . . .

to: Mrpe3-00078-F6-Wz.Pep check: 5990 from: 1 to: 192

TRANSLATE of: mrpe3-00078-f6-wz.ctg check: 4485 from: 70 to: 646
generated symbols 1 to: 192.
From: INTRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj: . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Blosum62.Cmp
CompCheck: 6430

Gap Weight: 12 Average Match: 2.912
Length Weight: 4 Average Mismatch: -2.003

Quality: 292 Length: 194
Ratio: 1.570 Gaps: 3
Percent Similarity: 49.730 Percent Identity: 40.541

Match display thresholds for the alignment(s):
| = IDENTITY
: = 2
. = 1

9808903.Pep x Mrpe3-00078-F6-Wz.Pep [redacted] 14:55 ..

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       .         .         .         .         .         .
     1 MIQKCLWLEILMGIFIAGILSLDCNLLNVHLRRVIWQNLRHLSSMSNSFP 50
       | | |||  |. ::|.   | .|    |:|    :|:: |||.  .||
     1 MILKYLWLVALVALYISPIQSQNC....VYLDHTILENMKLLSSIRTITP 46
       .         .         .         .         .         .
    51 VECLRENIAFELPQEFLQVTQPMKRDIKKAFYEMSLQAFNIFS.QHTFKY 99
       . ||::   || ||| | | | .|:|||  | .|  |  ||| . .
    47 LRCLKDTTDFEFPQEILLYVQHVKKDIKAVTYHISSLALIIFSLKDSISL 96
       .         .         .         .         .         .
   100 WKERHLKQIQIGLDQQAEYLNQCLEEDENENEDMKEMKENEMKPSEARVP 149
       | |..|. || .| :   :|: ::||.| :     :.       |
    97 ATEERLERIRSGLFKQVQQARECMVDEENKNIEEDSTSQHPHSEGFKAV. 145
       .         .         .         .         .         .
   150 QLSSLELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRCLYYFYK 193
         ||| :|| ||  ||  |||| |||.|| ||||||   |||
   146 ...YLELNKYFFRIRKFLVNKKYSFCAWKIVVEIRRCFSIFYK 186
```

To Page No.

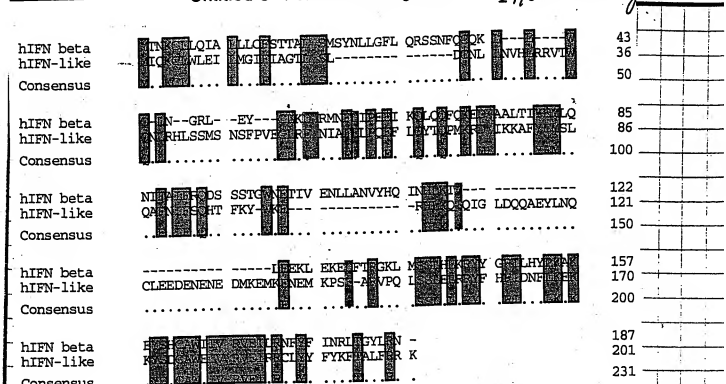
tnessed & Understood by me, | Date | Invented by | Date

Project No._____

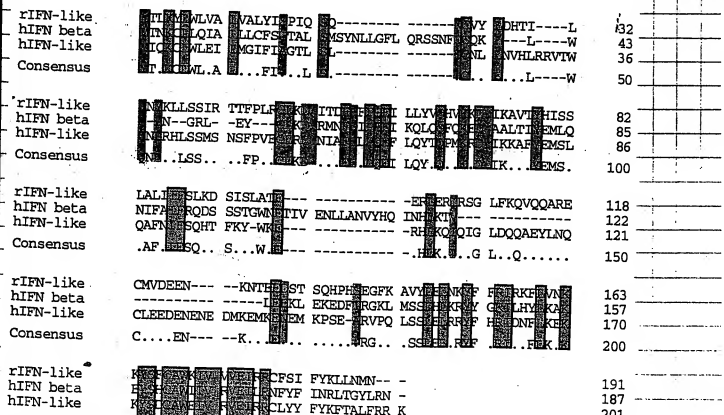
Book No._____ TITLE _____

17N

Untitled-5 Formatted Alignment 27% identity



Untitled-5 Formatted Alignment 17% identity



71

6/1198

IFN

NotI
BglI
EaeI BspI286I
HaeI Bpu1102I
HinPI BsiEI BanII NspHI BspI286I AlwNI Eam1105I
AAATGCAGG CCGCCGCGC CCTCAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CGTCAGTCT 700
TTTTACGTCC GGCGCGCGG GGAGTCGGG TTTAGAACAC TGTTTTGAGT GTGTACGGGT GCGACGGGTC GTGGACTTGA GGACCCCCT GCAGTCAGA
LysPheGlu lysArgAla1 aGlaGlnPro lysSerCysA spLysThrHi sThrCysPro ProCysPro A laProGlule uLeuGlyGly ProSerVal P
Factor Xa
NotI
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
10 20 30 40 50 60 70 80 90 100
BsiEI
NotI Bsp1286I
EagI BglI BanII NspHI AlwNI
"IFN" EaeI Bpu1102I BspI286I Eam1105I
AAATGCAGGG CTGACGCCA AATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC 700
TTTACGTCCC GACTGCGGT TAGAACACTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT GGACTTGAGG ACCCCCCTGG CAGTCAGAAG GAGAAGGGG
LysAlaAlaA laGluProLy sSerCysAsp LysThrHisT hrCysProPr oCysProAla ProGluLeuL euGlyGlyPr oSerValPhe leuPheProP
Factor Xa
Fc

Oligo No.: 1954-45 Purification: FOP
Researcher: wchen Standard Orde

Sequence
ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G
Display Expanded Sequence
Sal I + 2x stop codon (for prokaryotic

Oligo No.: 1954-46 Purification: FOP
Researcher: wchen Standard Order

Sequence
AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT
GAA TAG AGC T
Display Expanded Sequence
Not I + 2x Stop codon (for Prokaryotic

Oligo No.: 1954-47 Purification: FOPV
Researcher: wchen Standard Order

Sequence
CCC AAG CTT AGC ATG ATT CAA AAG TCT TTG TCG
Display Expanded Sequence
Hind III + ATG (a 5'

Oligo No.: 1954-48 Purification: FOPV
Researcher: wchen Standard Order

Sequence
Factor Xa
Not I R G F T
AAG GAA AAA AGC GGC CGC GCG GGC CTC GAT TT
CCT CCT GAA TAG AGC TGT AA
Not I Xa Fc
Display Expanded Sequence

Oligo No.: 1954-49 Purification: FOPV
Researcher: wchen. Standard Order

Sequence
Not I
AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG
AGC TGT AA
Display Expanded Sequence

From Page No.___

Chen, Wen

From: Schultz, Henry
Sent: Friday, [redacted] 5:15 PM
To: Chen, Wen
Subject: RE: human interferon like seq.

Wen - the human predicts cytokine strongly
scoreaacomp= -2.9 scoredipep= 19 ACCEPT (Probablility 91%).

The human is predicted to be signal peptide as follows:

MIQKCLWLEILMGIFIAGTLS cleavage LD.....etc

For the rat, the cytokine prediction is lost but the signal peptide is:

MTLKYLWLVA LVALYISPIQS cleavage QN....etc

Henry

From: Chen, Wen
Sent: Friday, [redacted] 4:02 PM
To: Schultz, Henry
Subject: human interferon like seq.

Henry: please help me look at the signal peptide for the human clone sequence. Thanks.

Wen Chen

Human sequence:
MIQKCLWLEI LMGIFIAGTL SLDCNLLNVH LRRVTWQNLR HLSSMSNSFP

51 VECLRENIAF ELPQEFLQYT QPMKRDIKKA FYEMSLQAFN IFSQHTFKYW

101 KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ

151 LSSLELRRYF HRIDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR

201 K

```
        10                  30                  50
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        70                  90                 110
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       130                 150                 170
GGATGTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
       190                 210                 230
TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
       250                 270                 290
TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
       310                 330                 350
CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAG
       370                 390                 410
TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAGC
       430                 450                 470
CTCAGAGGCAAAGGAAAGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
       490                 510                 530
CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
       550                 570                 590
TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
                                          MetSerThrLysProAs
       610                 630                 650
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pMetIleGlnLysCysLeuTrpLeuGluIleLeuMetGlyIlePheIleAlaGlyThrLe
       670                 690                 710
ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
uSerLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr
       730                 750                 770
ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
gHisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh
       790                 810                 830
TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
eGluLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl
       850                 870                 890
CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
aPheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr
       910                 930                 950
GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
pLysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGluTyrLeuAs
       970                 990                1010
CCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
nGlnCysLeuGluGluAspGluAsnGluAsnGluAspMetLysGluMetLysGluAsnGl
      1030                1050                1070
AATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
uMetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh
      1090                1110                1130
CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
eHisArgIleAspAsnPheLeuLysGluLysLysTyrSerAspCysAlaTrpGluIleVa
      1150                1170                1190
CGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
ArgValGluIleArgArgCysLeuTyrTyrPheTyrLysPheThrAlaLeuPheArgAr
      1210                1230                1250
AAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
```

To Page No.

Date

From Page No.___

gLysEnd

```
        1270                1290                1310
TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
        1330                1350                1370
ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
        1390                1410                1430
CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
        1450                1470                1490
TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
        1510                1530                1550
TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
        1570                1590                1610
ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
        1630                1650                1670
TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
        1690                1710                1730
TTTTCCCCCTCCTGCTCGGGGGGAAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
        1750                1770                1790
TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
        1810                1830                1850
ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
        1870                1890
GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT
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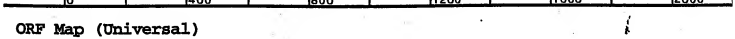
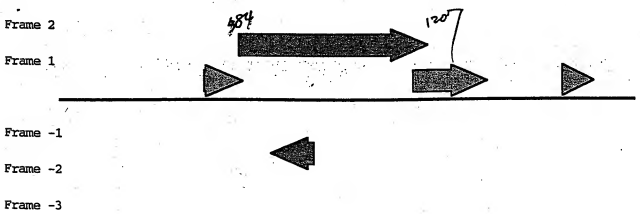


ORF Map (Universal)



One more ATG @ 684 in frame with ATG starting from 602.

To Page N

Witnessed & Understood by me. Date Invented by Date

D 1667
McDonnell
GN-0001

RESEARCH SUMMARY PAGE

Gene Name:

All Known Alias Gene Names:

Human: Zhwxc00-00001-a1 Rat: Agp-22423-a1	Member of the interferon family of proteins Name: Interferon-like protein.

Investigator(s):

Initial Date of Summary Preparation:

Duanzhi Wen, Andrew Welcher, Michael Kelley	Initial invention disclosure filed [redacted] This summary filled in on [redacted]

Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

Gene Nucleotide Sequence:

Human:

```
1    CGCGTACGTA AGCTTAATTT AACAAAATTG GAAAAACCTA AACTATACTG
51   TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAATGTCT
101   ATGATTAACT CAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA
151   AATATATTTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA
201   GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTCGC TTTCTGAAAT
251   ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT
301   CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTTCAGGGA
351   AAAAAAAAAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA
401   AAAAAAAAAA AAAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT
451   TGGTTAACTG TGAAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT
501   CAGGTATATA AAGGCACATG AAGGAAAACT CAAAACATCA TTGTCATATA
551   CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA
601   TATGATTCAA AAGTGTTTGT GGCTTGAGAT CCTTATGGGT ATATTCATTG
651   CTGGCACCCT ATCCCTGGAC TGTAACTTAC TGAACGTTCA CCTGAGAAGA
701   GTCACCTGGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTCC
751   TGTAGAATGT CTACGAGAAA ACATAGCTTT TGAGTTGCCC CAAGAGTTTC
801   TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA
851   ATGTCCCTAC AGGCCTTCAA CATCTTCAGC CAACACACCT TCAAATATTG
901   GAAAGAGAGA CACCTCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG
951   AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG
1001   AAAGAAATGA AAGAGAATGA GATGAAACCC TCAGAAGCCA GGGTCCCCCA
1051   GCTGAGCAGC CTGGAACTGA GGAGATATTT CCACAGGATA GACAATTTCC
1101   TGAAAGAAAA GAAATACAGT GACTGTGCCT GGGAGATTGT CCGAGTGGAA
1151   ATCAGAAGAT GTTTGTATTA CTTTTACAAA TTTACAGCTC TATTCAGGAG
1201   GAAATAAGGT ATATTTTTGG AATTAAAATT CCTTTTCCCT CCGAAATCTC
1251   TTTCTCCTTC TCCTCCTCCA TCTTCTTTTT AAGGATTGTT GTGCTGTCCT
1301   GTAAGCCTGT CCTCAGTTGG ACTGGTAGCC TCGGAACATC AGGGACACTC
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1351  ACCTCTCTAA GGAGAGGTAA TGCCAACCAT CCTCAGGGTG ACCAAGAGTC
1401  TCGTTAGAAA GTCTTTAAGA CATTTTTAAA GGAATAAGAT TCCCTCTCCG
1451  TCTTCTTCTA TTCTCTCTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT
1501  TGCTATATAT ACCACCTGTG GACTTCACCA AGACAATGGC TAGAGGATAG
1551  GGAGCAGAGA ATGTTGCAAA ATGGTAACAT TTCAATGACT TAACTGTTTT
1601  GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA
1651  TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG
1701  GGGAAAAAGG TTGACATTTC TGGCCCATTT CCTTCTCAGC TTGGTTTGTT
1751  TGAATTGATG CTTGTGGAAT GGTATTTCAT TACTTTAAGA GTGAAGATCC
1801  ATAGTGAAAT TGGATGGATG GTTGAATTAG ACGACCATTA AGCTTGGATC
1851  CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT

Rat:

   1 GGGTGTTGTA GATATTTTTC CTTTGGAAGA AATACTGAGC ACCAAGGCTG
  51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCTATACATT
 101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA
 151 AAACATGAAA CTTCTGAGCA GCATCAGGAC CACCTTTCCC TTAAGATGTC
 201 TAAAAGATAT CACGGATTTT GAGTTTCCTC AAGAGATTCT GCTGTACGTC
 251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT
 301 GGCGCTAATT ATTTTCAGTC TTAAAGACTC CATCTCCCTG GCGACAGAGG
 351 AACGCTTGGA ACGTATCAGA TCGGGACTTT TCAAACAAGT GCAGCAAGCT
 401 CGAGAGTGCA TGGTAGACGA GGAGAACAAG AACACGGAGG AGGACAGTAC
 451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA
 501 ACAAGTATTT CTTCAGAATC AGAAAGTTCC TGGTAAATAA GAAATACAGT
 551 TTCTGTGCCT GGAAGATTGT CGTCGTGGAA ATAAGAAGAT GTTTCAGTAT
 601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG
 651 AACTTAGATA GAAGTTGTGA CTGCTCAAAT GTCCCCAAGA ACGCTTGATT
 701 CTAAGGCTAT TGCGAGTCTG CTGCTACACA CTTCGGACGC AAGACTTTTC
 751 AAGGTCAGGG TTCAAGGTAG TACAGTCAAA GGAAGTCTTA TGTTAAGCAA
 801 AAGAAAAATT TCAGTGGAAA AGCTAGCAGA AATGTCAACT TGTCAAAAAA
 851 ACAACTTATG GATTATGGCA TTGACGTTAC TAGCAAAAAA AATAAAACAA
 901 AAAAAAACAA AAA
```

Gene Amino Acid Sequence:

Human:

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1  MSTKPDMIQK CLWLEILMGI FIAGTLSLDC NLLNVHLRRV TWQNLRHLSS
51  MSNSFPVECL RENIAFELPQ EFLQYTQPMK RDIKKAFYEM SLQAFNIFSQ
101  HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENNEDMK EMKENEMKPS
151  EARVPQLSSL ELRRYFHRID NFLKEKKYSD CAWEIVRVEI RRCLYYFYKF
201  TALFRRK*
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Rat:

```
1  MTLKYLWLVA LVALYISPIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
51  KDITDFEFPQ EILLYVQHVK KDIKAVTYHI SSLALTIFSL KDSISLATEE
101  RLERIRSGLF KQVQQARECM VDEENKMTEE DSTSQMPHSE GFKAVYLELN
151  KYFFRIRKPL VNKKYSFCAW KIVVVEIRRC FSIPYKLLNM N*
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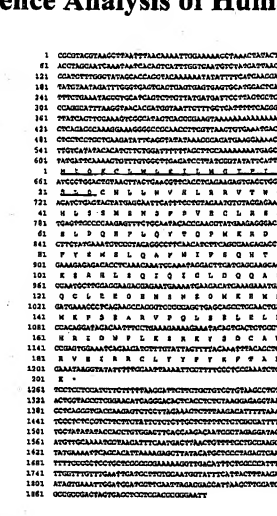
Figure Containing cDNA and Amino Acid Sequences:

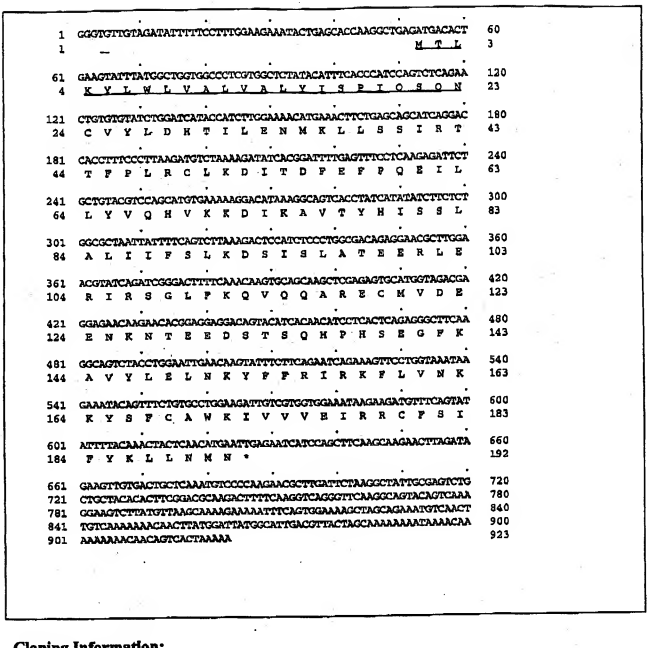
Human:

Sequence Analysis of Human IFN-novel



A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN-β.

Rat:



Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal 1 and Not 1 restriction sites.

Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFNa family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with $1x10^6$ human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50, 000 clonies / plate (*unnecessary information*). Nitrocellulose filters (*unnecessary information*) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

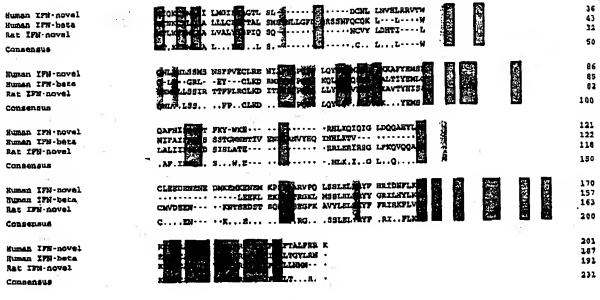
The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10μg/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate. Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1%SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1%SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (*unnecessary information*): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl2. Reaction condition: denature at 94 °C for 30sec, anneal at 60 °C for 30sec and elongate at 72 °C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100 °C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually $5x10^4$ cpm/μl.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

Homology of Multiple Gene Family Members:

Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN-β



- Human IFN-novel is most close to human IFN-β, with 30% identity. Four out of five cysteine residues are conserved between them.

Presence and Distribution of mRNA in Different Tissues:

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR *(conditions are not necessary - standard technology)* identified IFN-like mRNA in the following human tissues : pancreas, small intestine, prostate, uterus, thyroid, and placenta.

Recombinant Protein Expression:

Production of human and rat IFN-like protein in E. coli :

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

Production of human and rat IFN-like protein in a mammalian expression system:

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:
PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

Rat IFN-Like Molecule primers:

- IFN-Like molecule Fc-fusion:

1847-77 CCC AAG CTT ACC ATG ACA CTG AAG TAT TTA TG
Forward primer: Hind III site plus ATG
1847-78 AAG GAA AAA AGC GGC CGC ATT CAT GTT GAG TAG

- Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:
1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG
Reverse primer: Sal I site plus 2 stop codons (for pDSRα cloning).
1896-57 AAG GAA AAA AGC GGC CGC TCA TCA ATT CAT GTT GAG TAG
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN-like primers:

- Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G
Reverse prime: Sal I site plus 2 stop codons (for pDSRα cloning).
1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

- Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG
Forward primer: Hind III site with 1st ATG
1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C
Forward primer: Hind III site with 2nd ATG
1954-48 AAG GAA AAA AGC GGC CGC ***GCG GCC CTC GAT*** TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site, no stop codon with ***Factor Xa*** cleavage site and Fc fusion
1954-49 AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:
Rat:
Reaction Mixture: template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq polymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

Human interferon-like protein PCR conditions:
Reaction Mixture: template 20 ng, 1955-44 and 1954-45or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq plymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD⁻ cells.

Transfection:

- Protocol for transfeciton into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium phosphate transfection procedure, which has a commercially available kit.
-

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

Generate conditioned media containing recombinant protein.

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

Purification of human IFN-like-Fc

Conditioned media from CHO cells expressing hulFLM-Fc was thawed and 0.2μm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0 . After loading, the column was washed with PBS until the absorbence at A_{280} reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 an dimmediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing hulFLM-Fc were pooled and dialyzed into PBS and stored at -70°C.

Factor Xa cleavage of human IFN-like-Fc

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and hulFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM $CaCl_2$, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

Abs (available, ordered, proposed):

1. Polyclonal:
Polyclonal antibodies were prepared using both rat and human proteins produced in E. coli and CHO cells (from above) using standard immunological techniques. Antisera were positive for the proteins as determined by Western blot analysis (standard techniques)
2. Monoclonal:
None.
3. Peptides:
None.

Phenotype and/or Biological Activity:

1. Transgenic /	(pending / analyzed)

other	
Because the lack of a phenotype constitutes a 'negative' result no conclusions can be drawn from this experiment. Further testing will be required to determine any or all of IFN-like proteins' biological activities in vivo.	
2. *in vivo* assays:	(available, used, proposed)
Not done.	
3. *in vitro* assays:	(available, used, proposed)
Rat IFN-like Fc fusion protein treatment of several cell lines caused phosphorylation of some cellular proteins (unidentified).	

References:

Nothing specifically published on this gene. Lots of references for the interferon family.

Genomic DNA Sequence (i.e. including all introns and exons):

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

Ortholog DNA Sequences:

Human and rat sequences cloned.